Letters

Micropatterns of Protein and Conducting Polymer Molecules Fabricated by Layer-by-Layer Self-Assembly and Photolithography Techniques

Tingbing Cao,[†] Fang Wei,[‡] Xiaoming Jiao,[§] Jinyu Chen,[†] Wei Liao,[‡] Xinsheng Zhao,[‡] and Weixiao Cao*,[†]

College of Chemistry and Molecular Engineering, Peking University, Beijing 100871, China, State Key Laboratory of Molecular Dynamic and Stable Structures and Department of Chemical Biology, College of Chemistry, Peking University, Beijing 100871, China, and The Institute of Chemical Reagent, Beijing 100022, China

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An ultrathin film from polyaniline derivative (PAAA) and diazoresin photosensitive polymer (DR) was fabricated layer-by-layer (LBL) with a self-assembly technique. The micropatterns were achieved on LBL ultrathin film after UV exposure through a photomask. To directly characterize the patterned image, a fluorescent-labeled IgG was incorporated into the film, and a fair clear fluorescence image of the micropattern was achieved *using laser confocal fluorescence microscopy*. The controllable pattern of fluorescent-labeled IgG on a silicon surface may provide a simple and fast method for the immunoassay, and the conductivity of PAAA/DR film makes it possible to combine the electronics with the immunoassay.

Introduction

The ability to pattern surfaces on a microscopic scale is important for technological applications such as the manufacture of microelectronic circuits and digital storage media.[1] And there is presently enormous interest in patterning surfaces with micro- and nanometer resolution for both fundamental investigations and technological applications.[2−4] Designing polymer materials with controlled surface properties has long been an important issue in basic and applied research. The fabrication of polymer surfaces with nanoscale resolution patterns has emerged and has been an important challenge of nanoscience and nanotechnology.[5−9] Among different techniques for polymer materials nanofabrication, layer-by-layer (LBL) self-assembly, since its introduction by Decher et al.,[10,11] has become an increasingly popular technique because it is simple in procedure, easy to automate, and friendly to the environment.[12]

The driving forces for LBL self-assembly have been implemented including electrostatic interaction, hydrogen bonding, charge transfer, etc.[13−16] But most of those intermolecular forces are not stable enough toward polar solvents' etching or load-wearing. Also the self-assembly films fabricated using the LBL technique are usually ultrathin, and it is not quite easy to characterize them. A novel way to fabricate covalently attached multilayer films has been reported. The concept originated from the ionic or H-bonding self-assembly technique and then forming covalently attached multilayers by the photoreaction between the neighboring layers of the films.[17−19]

In this communication, an ultrathin film fabricated layer-by-layer from a conducting polymer, poly(aniline-*co*-*o*-anthranilic acid) (PAAA), and a photosensitive polymer, diazoresin (DR), a similar self-assembly film from conducting and photosensitive polymers has been described by Zhang et al.[20] The ultrathin film then was irradiated by UV light through a photomask. The irradiated areas of the film became insoluble, whereas the unirradiated areas kept their solubility and could be removed easily by alkaline water. In this way, the micropatterns are formed onto an ultrathin film in a mild condition which does not damage the conducting polymer.

[†] College of Chemistry and Molecular Engineering, Peking University.

[‡] State Key Laboratory of Molecular Dynamic and Stable Structures and Department of Chemical Biology, College of Chemistry, Peking University.

[§] The Institute of Chemical Reagent.

* Corresponding author.

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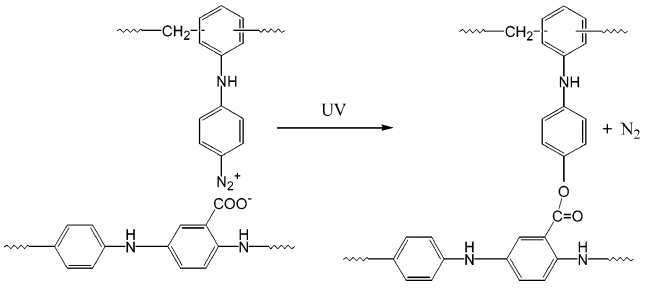
Scheme 1. The Schematic Representation of Bond Conversion from Ionic to Covalent Bond in the PAAA/DR Multiplayer Film under UV Irradiation



The ultrathin film now has been divided into two parts: hydrophilic (UV-irradiated areas) and hydrophobic (unirradiated areas). A fluorescence labeled IgG (immunoglublin G) protein, which is preferable to absorb onto the hydrophilic part of the film,[21] was used to reveal the pattern; we obtained a fairly clear fluorescence image using laser confocal fluorescence microscopy.

Experimental Section

Materials. The photosensitive polymer diazoresin (DR) was synthesized from diphenylamine-4-diazonium salt and paraformaldehyde in concentrated sulfuric acid,[22] $M_n \approx 2000$ g/mol, $\eta_{sp/c} = 0.12$ g/dL. Conducting polymer, poly(aniline-*co*-*o*-anthranilic acid) (PAAA) was synthesized from aniline and *o*-anthranilic acid according to ref 23. Goat anti-rabbit IgG molecules were purchased from Sigma, and the fluorescence was labeled by Jingke Biotech Company, China. Fluorescein isothiocyanate (FITC), phosphate-buffered saline (PBS), 1-(3-dimethylaminopropyl)-3-ethylcarbodiimide hydrochloride (EDAC), and *N*-hydroxysuccinimide (NHS) were purchased from Aldrich and used as received.

Process To Fabricate Self-Assembly Film. Silicon and quartz wafers were used as substrate to fabricate self-assembly film. Before use they were immersed in a fresh piranha solution (v/v = 1:3, 30% H_2O_2/98% H_2SO_4), heated until no bubbles were released, to create a clean and hydrophilic surface.

The silicon (or quartz) wafer was immersed at first in DR aqueous solution (0.2 mg/mL) for 5 min and then rinsed with deionized water and dried in flow air, followed by immersion in PAAA (0.5 mg/mL) weak alkaline aqueous solution (NH_4OH) for another 5 min, rinsed with deionized water, and dried to complete a fabrication cycle. In each cycle, a bilayer of DR−PAAA was deposited on the both sides of the substrate.

Photolithography. The silicon wafer modified with six bilayers of DR−PAAA multilayer film was exposed through a photomask under UV light from a medium-pressure mercury lamp. The exposure time is 15 s, and the irradiation intensity is 500 μW/cm^2 at 360 nm. After UV irradiation, the substrate was immersed in 0.1 M NaOH solution for 1 min and then was thoroughly washed by deionized water.

Fluorescent Image and Spectra. Fluorescence images were acquired with a microscope (Leica DMLM) equipped with a CCD cooled to −30 °C (CoolSNAP HQ Monochrome Photometrics, Roper Scientific, Tucson, AZ). Excitation light is the weak 490 nm line of a mercury lamp (50 W). H3 filters (Leica) were used to separate the excitation light from the signal. A 50× (Leica, air, NA = 0.75) microscope objective was used as both the excitation and collection optics. Fluorescence spectra were collected on a Raman microspectroscope (Renishaw System 1000, U.K.).

Results and Discussion

Diazoresin is a photosensitive polymer, which can achieve a covalent bond with negative groups of the film under UV irradiation. The bond conversion from ionic to covalent bond that takes place in the bilayers of the film composed of DR and PAAA, referring our previous studies,[24] can be schematically represented in Scheme 1.

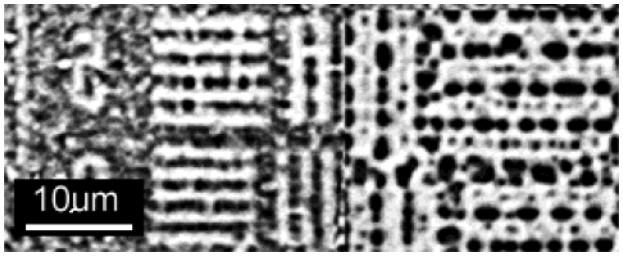


Figure 1. CCD image of the PAAA/DR patterned silicon surface after exposure to water vapor.

After UV irradiation through the photomask, the unirradiated part of the film, which is still linked electrostatically, can be removed using 0.1 M NaOH as developer. The aqueous NaOH solution selected as developer is based on the fact that the alkaline solution can achieve more clear images as compared with the common developers such as polar solvents, solvent−salt, or water−surfactant systems. The irradiated parts of the film are retained on the substrate, which are hydrophobic because the diazonium and carboxylic groups have been converted into ester bond as shown in Scheme 1. A faint image (Figure 1) was collected on an optic microscope as the substrate was treated by water vapor. We can find that the vapor, as micro water beads, coagulates on naked silicon, which is hydrophilic. No image can be found if the substrate is not treated by vapor. But the image was not clear and disappeared quickly as the water beads evaporated.

Because proteinarray technology provides a powerful and versatile tool for the proteome-scale analysis of protein functions, such as enzyme activity, protein−protein and protein−nucleic acid interaction, and small molecule−drug interactions.[25] A micropattern containing protein is of great interest in the field of biosensors and biochips. After getting a conjugated hydrophobic polymer image on a hydrophilic silicon surface, IgG (immunoglublin G) protein was used to adsorb on the hydrophilic part of the silicon surface.

Goat anti-rabbit IgG molecules were chosen to fulfill the protein pattern. The interaction of biological targets to compounds on the chip is best characterized in terms of kinetics and thermodynamics. The detection system must be extremely sensitive and capable of discriminating the surface-bound molecules from those free in solution. Laser confocal fluorescence microscopy meets these criteria.[26] Fluorescein isothiocyanate (FITC) labeled goat anti-rabbit IgG (FITC−IgG) can be easily absorbed to the hydrophilic parts, i.e., the naked silicon surface carrying a thin layer of SiO_2, which was previously activated for 1 h at 4 °C in phosphate-buffered saline (PBS) containing 5 mM 1-(3-dimethylaminopropyl)-3-ethylcarbodiimide hydrochloride (EDAC) and 0.5 mM *N*-hydroxysuccinimide (NHS) in order to anchor the FITC−IgG. And the PAAA/DR modified hydrophobic part is inertial to the FITC−IgG. A fairly clear fluorescence image of the pattern with defined resolution (the resolution of the photomask used is 0.8 μm) was achieved as shown in Figure 2. The bright regions represent the parts of surface modified by FITC−IgG and the dark regions represent the parts of PAAA/DR film.

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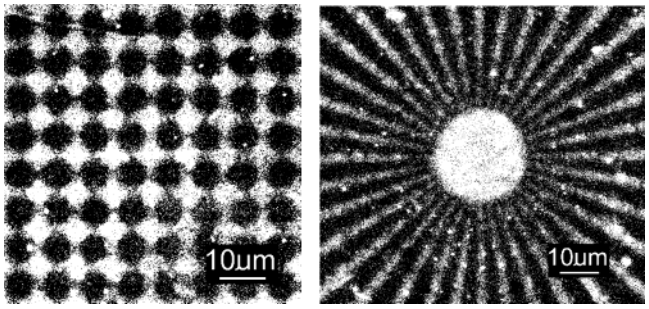

Figure 2. Fluorescence image of PAAA/DP micropattern on silicon surface obtained by FITC−IgG through a Leika microscope on Renishaw 1000 confocal Raman microspectroscope. The image is obtained by exciting of Hg Lamp passing through a set of filter and dichrome mirror (Leika, H3).

Since the fluorescence spectroscopy is sensitive enough to detect a monolayer of FITC−IgG antibody, it is a promising approach to reveal the micropattern from self-assembly films combining the FITC−IgG selected adsorption.

Figure 3 shows the fluorescence spectra of different parts on the surface obtained by laser confocal fluorescence microscopy. The FITC has a maximum emission at 550 nm (λ_{ex} = 514.7 nm). The hydrophobic parts of PAAA/DR film have little signal of fluorescence due to a minor absorbing of FITC−IgG, while the naked silicon parts modified by FITC-labeled IgG give intense fluorescence. The attachment of the FITC−IgG is stable enough to the washing of physical condition due to the chemical binding with the substrate surface. A more stringent wash procedure has been carried out, such as, 1 M ethanolamine, 0.2 M HCl, and 1 M NaOH used for the washing at the same time, and the discrimination is still acceptable. These results have proven that a stable modification of FITC−IgG on a hydrophilic surface of silicon is achieved.

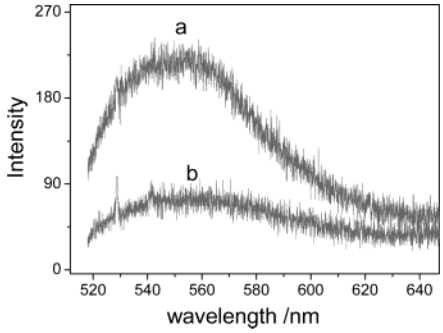

Figure 3. Fluorescence spectra of the IgG and PAAA/DR patterned surfaces: (a) IgG modified part and (b) PAAA/DR modified part. The sample is excited by a continuous wave laser (514.7 nm) on the Renishaw 1000 confocal Raman microspectroscope.

In conclusion, a micropattern comprised of PAAA/DR film and FITC−IgG protein was achieved on a silicon substrate. Using laser confocal fluorescence microscopy, we gathered a fair clear fluorescence image of the micropattern. The controllable pattern of fluorescent-labeled IgG on silicon surface may provide a simple and fast method for the immunoassay. The conductivity of PAAA/DR film makes it possible to combine the electronics with the immunoassay. The fluorescence labeled antibody should play an important role in the immunoassay of antigen−antibody recognition.

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